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Diagnosis of genital human papillomavirus (HPV) lesions in the male: Correlation of peniscopy, histology and in situ hybridisation

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Abstract

***Objective*—To assess the diagnostic criteria of genital HPV lesions in male sexual partners of HPV infected women.**

***Methods*—Peniscopically directed biopsy specimens (from 693 lesions in 300 men) were examined on light microscopy and in situ hybridisation (ISH) for HPV types 6,11,16,18,31,33 and 42. The predictive value of different histological criteria for ISH positivity was also evaluated using stepwise logistic regression analysis.**

***Results*—Flat HPV lesions were most accurately predicted by the punctuation pattern on peniscopy, giving the concordance between peniscopy and histology of 79·5% (66/83) and that between peniscopy and ISH of 56·6% (47/83). Diffuse acetowhite pattern disclosed a typical HPV lesion in only 17·8% (13/73), and HPV DNA was found in 11·0% (8/73) of cases. Of the 114 biopsy specimens from peniscopically healthy areas adjacent (0·5–1 cm) to the lesions, 93·0% (106/114) were normal on light microscopy, and HPV DNA was found in only 2·6%. Penile intraepithelial neoplasia (PIN) lesions were most frequently ISH positive, 81·1% (30/37), 50% showing HPV 16 and/or 18 DNA. Lesions classified as HPV-suspicious or nonspecific on light microscopy were HPV DNA-positive in 16·9% (11/65) and 8·1% (13/160), the frequency of high-risk HPV types being 3·1% and 1·3%, respectively. In logistic regression analysis, koilocytosis was the most powerful predictor of ISH-positivity in the flat lesions (without PIN), the risk ratio being 3·7.**

***Conclusion*—No conclusive peniscopic criteria for male HPV infections could be established, making histological evaluation mandatory. Care should be exercised in interpreting as HPV lesions the cases devoid of koilocytosis, HPV typing being essential in confirming the diagnosis in doubtful cases.**

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Introduction

There is substantial evidence linking HPV to female genital squamous cell cancer.[1 2 3] As a sexually transmitted disease, increasing interest has been focused on male HPV infections as well, the majority of which seem to appear subclinical and remain invisible to the naked eye.[4–8]

During the past few years, the role of acetic acid application and peniscopy has been emphasised in the diagnosis of male HPV lesions.[4 5 7 9–12] No uniformly accepted peniscopic criteria have been elaborated for the HPV-associated acetowhite lesions in the literature.[8] It is obvious, however, that acetowhite changes comprise a spectrum of histological abnormalities,[9–11] and only a part of the peniscopically HPV-suspect or even typical flat HPV lesions can be histologically confirmed as HPV infections.[10–13]

Recent advances in HPV detection techniques by hybridisation or PCR have added to the diagnostic difficulty.[14] HPV DNA can be disclosed in biopsies which do not meet the histological criteria of classical HPV lesions.[12 15–18] Conversely, lesions classified as condylomata on light microscopy can be negative by hybridisation tests.[12 15 18 19] Such findings can be attributed to the insensitivity of the hybridisation techniques or inappropriate DNA probes used.[13 20–22] On the other hand, HPV infections have a wide biological spectrum from a clinical disease to latent infections. In the latter, HPV should be confined to entirely normal epithelium, being detectable only by hybridisation or PCR.[14 18 21]

Histological diagnosis of condyloma is based on the presence of koilocytosis,[23–25] albeit papular lesions (pigmented papulosis, Bowenoid papulosis) commonly do not show these cytopathic changes of HPV.[3 8 18] Increased awareness of clinicians for HPV infections has resulted in an increased number of biopsies from the male genitals as well. The findings are not always easy to interpret, however, while containing subtle changes resembling those found in condylomas (i.e., acanthosis, hyper-keratosis, parakeratosis), but showing no conclusive evidence of HPV infection even if DNA detection methods are used.[8 18] To cope with this uncertainty, pathologists have started using ambiguous terms such as "suggestive of condyloma, borderline condyloma, subclinical HPV infection".[10 11 21 25 26] Such diagnostic terms with associated peniscopic abnormalities may be confusing for clinicians, leading to unnecessary ablative therapy in patients who actually never had an HPV-infection.

In the present study, an attempt was made to search for uniform diagnostic criteria of the male genital HPV infections, by comparing the peniscopic and histological findings with each other and as related to HPV DNA detection by in situ hybridisation (ISH) assay.

Material and methods

The material of the present study consists of 300 male sexual partners of women with histologically proven HPV infection, examined on the basis of abnormal Papanicolaou (PAP) smears. All males attended the Outpatient Department of Gynaecology and Obsterics, Kuopio University Hospital, voluntarily, and were subjected to peniscopic evaluation (after acetic acid) of the entire genital tract, as previously detailed.[10] From each patient, one or two biopsy samples were taken from peniscopically abnormal areas (altogether 579). In addition, 114 samples were taken from peniscopically healthy mucosa, adjacent to (0·5–1 cm) the biopsied lesion. Each biopsy specimen was fixed in 10% formalin and processed for routine histology and ISH according to routine procedures.

Peniscopic examination

All patients were examined by one of us (MIH) using an Olympus photocolposcope (peniscopy) at magnification 6–16x. Lesions were described according to their appearance as either (a) condyloma acuminatum (b) papular or (c) acetowhite flat lesions with or without sharp demarcation, elevated or having a punctuated capillary pattern. Papules were defined as well circumscribed elevated lesions with a smooth surface, and clearly distinct from condyloma acuminatum. Leukoplakia (white epithelium before acetic acid) and ulcers were also noted.

Histological examination

Altogether, 693 biopsy specimens were evaluated on light microscopy by a single pathologist (KS). The histological findings were classified into one of the following categories: 1) condyloma acuminatum, 2) flat condyloma, 3) papulosis 4) suspicious for HPV, 5) nonspecific, and 6) healthy mucosa. The criteria used in categorising the HPV lesions according to their light microscopic appearance have been published previously.[3 8 18] Lesions with subtle changes, such as mild acanthosis or parakeratosis, no typical koilocytes, and minimal or no cellular atypia, were classified as suspicious for HPV (equivalent to subclinical HPV infection?). Findings devoid of any morphological evidence for HPV were classified as nonspecific, that is, usually inflammation, hyper- or parakeratosis.

Table 1 Histological findings behind the peniscopic patterns

		Histology			
Peniscopic Appearance	*No.*	*Typical HPV No. (%)*	*Susp. HPV No. (%)*	*Nonsp. No. (%)*	*Healthy No. (%)*
Acuminatum	57	53 (93·0)	0	3 (5·3)	1 (1·8)
Papular	87	52 (59·8)	6 (6·9)	23 (26·4)	6 (6·9)
Acetowhite	*419*	*222 (53·0)*	*56 (13·4)*	*120(28·6)*	*21 (5·0)*
–diffuse	73	13 (17·8)	7 (9·6)	46 (63·0)	7 (9·6)
–well demarc.	81	36 (44·4)	14 (17·3)	27 (33·3)	4 (4·9)
– + elevated	182	107 (58·8)	25 (13·7)	40 (22·0)	10 (5·5)
– + punctuation	83	66 (79·5)	10 (12·0)	7 (8·4)	0
Leukoplakia	2	1 (50·0)	0	1 (50·0)	0
Ulceration	12	4 (33·3)	0	7 (58·3)	1 (8·3)
Herpes	2	0	0	2 (100)	0
Healthy	114	1 (0·9)	3 (2·6)	4 (3·5)	106 (93·0)
Total	693	333 (48·1)	65 (9·4)	160 (23·1)	135 (19·5)

For the present analysis, all biopsy samples were scored according to 10 different histological characteristics previously associated with HPV infections; koilocytosis, cell vacuolisation, papillomatosis, acanthosis, hyperkeratosis, parakeratosis, dyskeratosis, inflammation, hypergranulosis and dysplasia. Penile intraepithelial neoplasia (PIN), characterised by disorganised epithelial layers and cellular polarity, nuclear atypia, and abnormal mitotic figures, was graded according to the principles followed for intraepithelial neoplasia in the female genital tract as defined previously.[3 8 18]

In situ hybridisation (ISH)

All biopsies were analysed for the presence of HPV DNA using the ISH applied on paraffin sections. The ISH technique routinely used in our laboratory is based on biotin-labelled DNA probes for HPV types 6, 11, 16, 18, 31, 33 and 42, as previously detailed.[20 27 28]

Statistical methods

To analyse the correlations between different histological criteria and ISH, Pearson's correlation coefficient was used. Distribution of class variables were tested with the chi square test or with Fischer's exact test when adequate. The value of the different histological characteristics to predict HPV DNA-positivity in the flat lesions (both typical HPV, suspicious HPV, and nonspecific ones) was assessed with logistic regression analysis used in a stepwise manner; variable selection was based on likelihood ratio statistics. Analysis was carried out with the following variables as predictors: koilocytosis, papillomatosis, acanthosis, vacuolisation, parakeratosis, hyperkeratosis, dyskeratosis, hypergranulosis and inflammatory changes. All calculations were carried out with the SPSSPC v4.01 computer programme.

Results

Histological findings behind the different peniscopic patterns are summarised in table 1. Condyloma acuminatum was highly accurately diagnosed on peniscopy (93%), and a similar accuracy (93%) was obtained when the biopsies from peniscopically healthy areas were assessed on light microscopy. Diffuse acetowhite lesions were poor predictors of HPV infection, a typical lesion being confirmed in only 17·8% (13/73) of cases. However, sharp demarcation, elevation and punctuation pattern of the acetowhite lesions increased the HPV-predictability of peniscopy to 79·5% (66/83). Whereas 25·3% of the flat lesions with punctuation pattern disclosed a PIN, only 1·4% of the diffuse acetowhite lesions were due to PIN. Of the papular lesions, most of the smallest ones (<5mm, often on the shaft), appeared to be either nonspecific or flat condylomas. Instead, all the pigmented papules were typical HPV lesions, seven of them being Bowenoid papulosis. Two lesions were classified as leukoplakia; just another of them showed typical

Table 2 HPV DNA in lesions with different peniscopic patterns

Peniscopy	No.	ISH-positive No. (%)	ISH-negative No. (%)
Acuminatum	57	48(84·2)	9 (15·8)
Papular	87	32 (36·8)	55 (63·2)
Acetowhite	419	100 (23·9)	319 (76·1)
–diffuse	73	8 (11·0)	65 (89·0)
–well demarc.	81	9 (11·1)	72 (88·9)
– + elevated	182	36 (19·8)	146 (80·2)
– + punctuation	83	47 (56·6)	36 (43·4)
Leukoplakia	2	0	2 (100)
Ulceration	12	1 (8·3)	11 (91·7)
Herpes	2	0	2 (100)
Healthy	114	3 (2·6)	111 (97·4)
Total	693	184 (26·6)	509 (73·4)

features for HPV on light microscopy.

The detection of HPV DNA by ISH as related to peniscopic patterns is shown in table 2. Condylomata acuminata were almost invariably HPV DNA-positive (84·2%), whereas peniscopically normal epithelium showed HPV DNA in only 2·6% of cases (3/114). Diffuse acetowhite flat lesions were mostly ISH-negative 89·0% (65/73), whereas 56·6% of the well demarcated, elevated lesions with punctuation pattern were ISH-positive (47/83). The distribution of different flat lesions between ISH positive and negative groups was statistically significant ($p < 0·001$).

HPV types in different penile lesions are depicted in table 3. The concordance between histology and HPV typing was equally high in condylomata acuminata and PIN, 81·1%, but the distribution of high and low risk types was significantly different ($p < 0·001$). The high risk types 16 and 18 were confined to PIN and typical flat lesions in 80% (28/35) and never found in condyloma acuminata. On the other hand, HPV types 6 and 11 were found in 77·4% (41/53) of condylomata acuminata and in 33·3% (15/45) of papulosis lesions. Most interestingly, HPV 6 and/or 11 were detected also in 10·8% of PIN lesions (4/37). HPV detection rate was substantial (16·9%) in histologically HPV-suspicious lesions, but markedly lower (8·1%) in those classified as nonspecific. Histologically normal epithelium adjacent to the lesions showed HPV DNA in 6·7% (9/135) of cases.

The distribution of different histological criteria in ISH-positive and ISH-negative biopsies is shown in table 4. Only hyperkeratosis and inflammatory changes were practically identical in these two groups, all the other histological characteristics being significantly ($p < 0·001$) different in HPV-positive and HPV-negative lesions. When analysed using Pearson's correlation coefficient, the histological features correlating significantly with the HPV-positivity were koilocytosis ($r = 0·37$, $p < 0·001$), papillomatosis ($r = 0·25$, $p < 0·001$), acanthosis ($r = 0·18$, $p < 0·001$), parakeratosis ($r = 0·15$, $p < 0·001$), and hypergranulosis ($r = 0·12$, $p < 0·01$)(data not shown in tables). In stepwise logistic regression analysis, koilocytosis, papillomatosis and hypergranulosis were selected as independent predictors for HPV-positivity. Koilocytosis was the single most powerful predictor, the risk ratio (RR) being 3·7.

The influence of lesion size and location on HPV-detectability on histology and ISH is summarized in table 5 (altogether 579 lesions). The largest sharply demarcated lesions were most frequently typical HPV lesions on light microscopy, and HPV DNA-positive on ISH as well. Confluent lesions consisted of huge exophytic condylomas sometimes with adjacent flat lesions, or were clinically consistent with balanoposthitis and prominent infection. Histologically typical HPV lesions had distinct sites of predilection in the male genitalia; urethral and meatal lesions were most frequently typical HPV, 100% (15/15) and 70% (7/10), respectively, whereas lesions on the glans and in the

Table 3 HPV DNA in different penile lesions

Histology	No	ISH-positive, No. (%)			
		No (%)	6/11	16/18	31,33,42
PIN*	37	30 (81·1)	4 (10·8)	15 (40·5)	11 (29·7)
Acuminatum	53	43 (81·1)	41 (77·4)	0	2 (3·8)
Papular	45	22 (48·9)	15 (33·3)	3 (6·7)	4 (8·9)
Flat	198	60 (30·3)	32 (16·2)	13 (6·6)	15 (7·6)
Susp flat	65	11 (16·9)	5 (7·7)	2 (3·1)	4 (6·2)
Nonspef	160	13 (8·1)	8 (5·0)	2 (1·3)	3 (1·9)
Healthy	135	9 (6·7)	6 (4·4)	0	3 (2·2)
Total	693	188 (27·2)	111 (16·0)	35 (5·1)	42 (6·1)

*7 Bowenoid papulosis, 30 flat lesions

Table 4 Morphological characteristics related to detection of HPV DNA by ISH

Morphological Feature	Positive Biopsies N = 188	ISH- Negative Biopsies N = 505	ISH-
Non-PIN-lesions	No. (%)	No. (%)	
—papillomatosis	21 (11·2)	4 (0·8)	$p < 0·001$
—koilocytosis	127 (67·6)	135 (26·7)	$p < 0·001$
—vacualisation	13 (6·9)	74 (14·7)	$p = 0·001$
—acanthosis	116 (61·7)	204 (40·4)	$p < 0·001$
—parakeratosis	68 (36·2)	110 (21·8)	$p < 0·001$
—hyperkeratosis	50 (26·6)	160 (31·7)	$p = 0·21$
—dyskeratosis	3 (1·6)	4 (0·8)	$p < 0·001$
—inflammation	22 (11·7)	80 (15·8)	$p = 0·18$
—hypergranulosis	16 (8·5)	14 (2·8)	$p = 0·003$
PIN*	30 (16·0)	7 (1·4)	$p < 0·001$
healthy	9 (4·8)	126 (25·0)	$p < 0·001$

PIN*: 7 Bowenoid papulosis and 30 flat lesions

Table 5 Lesion size and location as related to histology and HPV DNA detection

(a) Size of lesion	Total series N (%)	Histologically typical lesions N (%)	ISH-positive lesions N (%)
1–2mm	207 (35·8)	105 (50·7)	42 (20·3)
2–10mm	259 (44·7)	165 (63·7)	86 (33·2)
>10mm	58 (10·1)	46 (79·3)	29 (50·0)
Confluent	54 (9·3)	25 (46·3)	17 (31·5)
(b) Site of lesion			
Urethra	15 (2·6)	15 (100)	12 (80·0)
Meatus	10 (1·7)	7 (70·0)	4 (40·0)
Glans	13 (2·2)	3 (23·1)	2 (15·4)
Sulcus	60 (10·4)	25 (41·7)	16 (26·7)
Prepuce	225 (38·9)	137 (60·9)	75 (33·3)
Frenulum	67 (11·6)	24 (35·8)	9 (13·4)
Shaft	148 (25·6)	95 (64·2)	45 (30·4)
Scrotum	14 (2·4)	6 (42·9)	3 (21·4)
Anus	10 (1·7)	7 (70·0)	2 (20·0)
Groins	10 (1·7)	6 (60·0)	7 (70·0)
Pubic	6 (1·0)	5 (83·3)	2 (33·3)

Table 6 HPV-types related to peniscopic and morphologic evidence for HPV

HPV type	No. of Cases	Peniscopically Typical HPV* No. (%)	Histologically Typical HPV No. (%)
6/11	111	92 (82·9)	92 (82·9)
16/18	35	28 (80·0)	31 (88·6)
31,33,42	42	33 (78·6)	25 (59·5)
ISH-	505	163 (32·3)	180 (35·6)

* = exophytic warts and flat acetowhite sharply demarcated lesions

frenulum were more often nonspecific and not consistent with HPV infection, 75·0% and 64·2%, respectively. This distribution of HPV lesions closely correlated with the detectability of HPV DNA by ISH, being highest in the lesions of urethra and meatus, and lowest in those of the glans and frenulum (table 5).

Of the ISH-positive lesions with high risk types 16 and 18, 20% were not typical for HPV peniscopically (were diffuse acetowhite lesions). On the other hand, the high-risk types were never found from the epithelium classified as normal on peniscopy. Similarly, 11·4% of the biopsies containing high-risk types were not histologically typical for HPV. Some 30% of ISH-negative lesions were peniscopically and histologically typical for HPV, as shown in table 6.

Discussion

In the proper diagnosis of male genital lesions, it is essential to assess what proportion of the visible lesions in men known to be exposed to the virus (that is, sexual partners of HPV-infected women) are HPV-related. In the present study, peniscopical and histological criteria used in the diagnosis of genital HPV infections were evaluated using the HPV DNA detection by ISH as a reference.

The role of peniscopy in HPV diagnosis has been discussed in an increasing number of recent reports,[4-12 29] but reliable diagnostic criteria have not been elaborated as yet. Many studies have emphasised, however, that the most consistent single pattern of penile flat HPV lesions is the presence of a well demarcated, slightly elevated acetowhite epithelium in which a capillary punctuation can be distinguished.[5 9 12] This is consistent with our experience as well. When examined on light microscopy, none of the lesions with the above pattern were normal, but instead, 91·6% of them were morphologically typical or suspicious HPV (table 1), and 56·6% showed HPV DNA (table 2). If, however, the punctuation pattern of capillary had been the demanded criteria for obvious flat HPV lesions evaluated by peniscopy, only 22·7% (66/222) of the lesions classified as typical for HPV on light microscopy, had been detected. But by counting all the sharply demarcated lesions (elevated or not) with punctuation, 94·1% of histologically typical HPV lesions were found.

In some recent reports,[21 29] the question has been raised on the usefulness of peniscopy, while especially the smallest lesions seem to be often histologically nonspecific and HPV DNA is not found. In our study, the lesions ranging from 1–2mm, were histologically typical flat lesions in 50·7% of cases and ISH-positivity was found in 20·3%. Thus, these small flat lesions should not be neglected if correct diagnosis is to be reached. However, in the future it would be more rewarding to classify the papules according to their size; the smallest ones (<5mm) usually being nonspecific or suspicious flat lesions on light microscopy. On the other hand, pigmented papules almost invariably showed histological features of PIN as has been reported by others.[4 9] The site of the lesion can only suggest the possible diagnosis.

Although we were not able to establish conclusive peniscopic criteria for flat HPV lesions, we do stress the importance of peniscopy, because even small exophytic warts may be overlooked without adequate magnification and a good light source. The reliability of peniscopy in evaluating the extent of HPV infection and disclosing the disease is well established by our results; 93·0% of the peniscopically healthy areas adjacent to the lesion were histologically normal (table 1). Only 2·6% of them were ISH-positive, but never contained HPV types 16 and 18. However, the use of acetic acid without peniscopy must be cautioned, because of a great number of false positive results, as reported before.[15 21 29 26] Another question is whether acetic acid application is of clinical use in searching for subclinical (mostly symptomless) lesions in the male genitalia.

The present results clearly indicate that no major problems are encountered in making the correct diagnosis of classical warts and intraepithelial neoplasia lesions. There was a good correlation between histology and ISH in condyloma acuminatum and PIN lesions, both being ISH-positive in close to 80% of cases. The distribution of different HPV types was consistent with the previous reports,[4 9 12 15 30] the high-risk types being most frequent in PIN lesions. The correlation of histology and ISH in the flat N-PIN lesions was more poor, however, since only 30% of even typical flat lesions showed HPV DNA. This is in agreement with the earlier findings of Nuovo *et al*, who reported that lesions clinically resembling condylomata frequently did not contain detectable HPV DNA when koilocytotic atypia and multinucleation were absent.[21] Similar problems are encountered while evaluating the flat vulvar lesions as well.[21] Noteworthy was the observation that histologically nonspecific lesions were ISH-positive in 8·1% of cases, showing the high-risk HPV types in 1·3%. This emphasises the difficulty in making the morphological diagnosis of subclinical HPV infections, as recently pointed out.[18]

An attempt was also made to evaluate the predictive value of histologic criteria used in diagnosis of HPV-associated NPIN-lesions (as confirmed by ISH). There was a statisti-

cally significant difference in the prevalence of most of these histological criteria between the ISH-positive and ISH-negative lesions (table 4). However, the model constructed on the basis of our stepwise logistic regression analysis to predict the ISH-positivity in flat lesions, is hardly useful; although it could predict 80% of the specimens correctly, only 8% of the ISH-positive findings were correct positive ones.

In the present material, the biopsy samples subjected to light microscopic analysis were identical to those analysed for HPV DNA, thus providing a direct comparison of the histological features with the HPV detection. Seven of the most common genital HPV types were analysed by ISH. Using different hybridisation methods together or with PCR, the HPV-detection rate might have been higher.[12 13 16 17] It seems likely, however, that no clinically important lesions remained undetected, as suggested by the analysis of PIN and condyloma acuminatum lesions which were invariably ISH-positive. Interestingly, we did not notice any double infections when single lesions were analysed, in contrast to some previous findings.[31 32] However, seven patients with double infections were found when biopsies from different lesions were taken into account, that is, one from an exophytic wart and another from an adjacent flat lesion.

We can conclude that peniscopy is a clinically useful and mandatory diagnostic tool to be included in the satisfactory clinical evaluation of any patient with suspected HPV infection. This is so notwithstanding that conclusive criteria for peniscopically flat lesions cannot be given. Sharp demarcation of the lesions is highly suspicious for HPV infection, whereas a punctuated capillary pattern frequently suggests a PIN. Histological evaluation is needed to confirm the diagnosis. However, caution should be exercised while interpreting the nonclassic histological criteria (without typical koilocytosis) as an HPV lesion, viral typing being required to confirm the diagnosis in doubtful cases. The role of the clinician is to be emphasised; he or she must be able to conclude the case on the basis of the results from different diagnostic methods. According to our experience, a restrictive attitude to the therapy of male genital lesions seems feasible, especially when there are only subclinical (peniscopically detected) lesions without symptoms. This is particularly recommended if the lesions are not typically HPV-related or no high risk types have been found. It should be remembered that many therapies are quite ablative and there seems not to be very effective methods for treating flat lesions, though laser therapy seems to be most suitable. Many lesions also seem to disappear while just waiting (data not shown) and the malignant transformation is actually very rare. However, exophytic warts are better treated (for at least cosmetic reasons), as also men with symptoms. By following peniscopically symptomless patients to see if their lesions progress or regress we could also learn more about the biological behaviour of the male genital infection.

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